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Development of anti-polysaccharide antibodies in asplenic children

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SUMMARY

Splenectomized patients are highly susceptible to infections with capsulated bacteria and an impaired response to vaccination with bacterial polysaccharides has frequently been observed in these individuals. Based partly on experimental animal data, an important role for the spleen in the production of specific anti-carbohydrate antibodies, i.e. mostly IgG2 in normal adults, has been suggested. We therefore determined the immunoglobulin class and subclass pattern of serum antibodies from asplenic patients against protein and carbohydrate antigens. Normal levels of total serum IgM, IgG2 and specific IgM and IgG2 anti-polysaccharide antibodies were observed, suggesting only a minor role for the spleen in determining the antibody repertoire. The data imply that the impaired phagocytic capacity and/or the inability to mount a sufficiently rapid antibody response are the main factors underlying the increased susceptibility to bacterial infections noted in these patients.

Keywords asplenia immunity immunoglobulin subclasses

INTRODUCTION

Splenectomized patients are unduly susceptible to bacterial infections and most of the overwhelming infections observed are due to capsulated bacteria such as *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae*. An increased incidence of similar types of infections has also been noted in patients with congenital asplenia.

In experimental animals, the marginal zone of the spleen contains a unique subset of B lymphocytes which are characterized both by distinct surface markers and functional capacity (Gray *et al.*, 1984; 1985). These cells appear to be specifically involved in the response against thymus-independent antigens such as polysaccharides and the absence of this particular subpopulation may help explain the impaired response to vaccination with bacterial capsular polysaccharides observed in splenectomized patients (Amman *et al.*, 1977; Weitzman *et al.*, 1977; Siber *et al.*, 1978; Minor, Schiffman & McIntosh, 1979; Hosea *et al.*, 1981; Pedersen, Henricksen & Schiffman, 1982; Amlot & Hayes, 1985; Oldfield *et al.*, 1985). In some of these studies, the underlying disease or concomitant immunosuppression may have contributed to the impaired response and a number of additional reports do in fact suggest that the response in patients splenectomized due to trauma is normal (Amman *et al.*, 1977; Sullivan *et al.*, 1978; Giebink *et al.*, 1981; Johansen & Pedersen, 1982; Pedersen, Nielsen & Ellergaard, 1982), an effect which is believed to be due to the seeding from the spleen of cells having responded to pneumococcal polysaccharides already prior to splenectomy (Amlot & Hayes, 1985).

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In addition to specific IgM antibodies, responses against polysaccharides are normally restricted to the IgG1 subclass in children with a gradual shift to IgG2 as the immune system matures (for review see Hammarström & Smith, 1986). Since a substantial proportion of B cells secreting anti-polysaccharide antibodies may be expected to be lacking in asplenic patients, a pertubed pattern of serum antibodies against carbohydrate antigens would ensue. We therefore undertook a study of the subclass distribution of specific antibodies in patients with congenital asplenia and patients who had been splenectomized before bone-marrow reconstitution.

MATERIALS AND METHODS

Measurement of serum class and IgG subclass levels. Sera were collected and stored at −20°C until analysed. Immunoglobulin class and subclass levels were determined in immunodiffusion using commercially available polyclonal reagents (for IgM, IgG and IgA) (Behringwerke, Marburg, West Germany) or monoclonal reagents (for the IgG subclasses) (Seward Laboratories, London, England). Data from the four asplenic patients analysed are given in Table 1. Antibodies against pneumococcal polysaccharides in these patients are assumed to be due to natural exposure except in the first patient who was vaccinated with Pneumovax.

Subclass pattern of specific antibodies. The IgG subclass distribution of antibodies against a variety of antigens was tested in ELISA as previously described in detail (Persson, Hammarström & Smith, 1985). Briefly, antigens (pneumococcal capsular polysaccharides type 3, 6A and 19F and 23) (a gift from MSD, Mechelem, Belgium), teichoic acid (batch 8a) from *S. aureus* (a gift from Dr R. Möllby, National Bacteriological Laboratory, Stockholm, Sweden), tetanus toxoid (a gift from Dr M. Fall-Persson, National Bacteriological Laboratory, Stockholm, Sweden) and outer membrane protein from H. influenzae (a gift from Dr I. Allan, Oxford, England) were coated onto microtitre plates. After incubation with patient serum samples (diluted 1:100), commercially available monoclonal antibodies against the various IgG subclasses (Seward Laboratories, London, England) were added followed by a rabbit anti-mouse (Dakopatts, Glostrup, Denmark) and alkaline phosphatase conjugated goat anti-rabbit immunoglobulin antibodies (Sigma Chemical Company, St Louis, MO, USA). For determination of levels of specific IgM antibody levels, polyclonal rabbit anti-μ-antibodies (DAKO, Glostrup, Denmark) were added followed by the same developing antiserum as for the IgG subclass assay.

RESULTS

Normal serum levels of IgM and the tested IgG subclasses were present in all four patients (Table 2). Comparable levels of specific IgM antibodies (judged by similar absorbance levels in ELISA) were

Table 1. Diagnosis of asplenic patients

Case	Sex	Diagnosis
B.B.*	M	Congenital asplenia
I-M.S	F	Congenital asplenia
A.A.	F	Chronic myelogenous leukaemia (splenectomized before bone-marrow transplantation)
L.J.	F	Gaucher's disease (splenectomized before bone-marrow transplantation)

* Vaccinated (Pneumovax) at two years of age. Serum samples were obtained at 10 months of age and 3 months post vaccination.

Table 2. Immunoglobulin levels in asplenic patients

Case	Age	IgM	IgG1	IgG2	IgG3	IgG4	IgA
B.B.	1	0·7	4·9	0·8	0·7	NT	0·6
	2	0·3	6·4	0·7	0·3	0·29	1·3
I-M.S	11	1·0	5·2	1·0	0·5	0·07	0·6
A.A.	(12)*	1·0	6·2	2·5	0·3	0·70	1·4
L.J.	(9)*	2·0	5·4	0·4	0·3	0·06	0·1

Levels are given as g/l. All values are within the normal range (corrected for age after transplantation) (Oxelius, 1979).

NT, Not tested.

* Age at transplantation. Serum samples were obtained 2 years after grafting.

Table 3. Levels of specific IgM levels in asplenic children

Donor	Age	PPS 3*	PPS 6A*	PPS 19F*	Teichoic acid†	Tetanus toxoid	*H. influenzae*‡
B.B.	2	0·37	0·16	0·06	0·33	0·13	0·13
I-M.S.	11	0·58	0·25	0·36	0·41	0·51	0·24
A.A.	(12)§	0·47	0·22	0·08	0·40	0·31	0·23
L.J.	(9)§	0·40	0·24	0·17	0·48	0·20	0·20
Control	2	0·47	0·35	0·19	0·43	0·26	0·27
Control	Adult	0·40	0·32	0·09	0·41	0·32	0·24
Control	Adult	0·47	0·33	0·16	0·39	0·26	0·30
CVID¶	Adult	0·02	0·01	0·03	0·06	0·04	0·03

Values are given as net absorbance levels after 10 min.

* Pneumococcal capsular polysaccharides of various serotypes.

† Batch 8.

‡ Outer membrane protein from *Haemophilus influenzae*.

§ Age at the time of transplantation. Serum samples were obtained 2 years after transplantation.

¶ Common variable immunodeficiency (hypogammaglobulinemia).

found in patients and controls (Table 3). In addition, when corrected for age, the expected subclass pattern of antibodies against both protein (Table 4) and carbohydrate antigens was observed (Table 5) (data not shown for antibodies against pneumococcal polysaccharide types 3 and 23).

DISCUSSION

The risk of systemic bacterial infections in splenectomized patients has previously been well documented. The increased disease susceptibility is not restricted to patients with asplenia due to surgical removal of the organ but is also evident in patients with a mere functional impairment of the spleen.

Antibodies to the polysaccharide capsule of certain bacterial strains such as pneumococci play an important role in the defense against infection. In mice, antipneumococcal antibodies of the IgG

Table 4. Subclass pattern of antibodies against protein antigens in asplenic patients

			Tetanus toxoid				*H. influenzae**			
Exp.	Case	Age	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
1	B.B.	2	0·42	0·03	0·04	0·23	0·19	0·00	0·02	0·01
	Control	1	0·99	0·05	0·07	0·24	0·20	0·00	0·04	0·00
	Control	2	0·20	0·03	0·03	0·03	0·33	0·00	0·14	0·02
2	I-M.S.	11	1·52	0·01	0·19	0·17	0·78	0·04	0·12	0·02
	A.A.	(12)†	1·21	0·02	0·11	0·25	0·20	0·01	0·01	0·03
	Control	Adult	1·16	0·00	0·06	0·15	0·47	0·02	0·06	0·02
	Control	Adult	1·15	0·00	0·03	0·08	0·55	0·08	0·08	0·06
3	L.J.	(9)†	0·05	0·00	0·03	0·01	0·32	0·00	0·00	0·00
	Control	11	1·36	0·02	0·00	0·15	0·63	0·19	0·08	0·00
	Control	16	1·07	0·05	0·01	0·09	0·94	0·09	0·00	0·00

Results are given as net absorbance values after 10–30 min. Data on a large number of age matched controls are given in Freijd *et al.* (1984) and Hammarström *et al.* (1985).

* Outer membrane protein from *Haemophilus influenzae*.

† Age at the time of transplantation. Serum samples were obtained 2 years after transplantation.

Table 5. Subclass pattern of antibodies against polysaccharide antigens in asplenic patients

			PPS 6A				PPS 19F				Teichoic acid*			
Exp.	Case	Age	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
1	B.B.	2	0·16	0·20	0·02	0·03	0·27	0·07	0·02	0·02	0·57	0·05	0·10	0·04
	Control	1	0·29	0·10	0·05	0·04	0·16	0·04	0·02	0·00	0·32	0·06	0·05	0·05
	Control	2	0·21	0·06	0·07	0·03	0·16	0·03	0·06	0·01	0·96	0·04	0·03	0·04
2	I-M.S.	11	0·41	1·76	0·01	0·09	0·27	0·20	0·04	0·01	0·10	0·39	0·01	0·02
	A.A.	(12)†	0·31	1·49	0·03	0·06	0·13	0·11	0·02	0·02	0·03	0·69	0·00	0·02
	Control	Adult	0·50	1·32	0·05	0·05	0·15	0·31	0·02	0·03	0·02	0·53	0·00	0·00
	Control	Adult	0·05	1·70	0·03	0·11	0·07	0·10	0·01	0·01	0·04	0·68	0·03	0·04
3	L.J.	(9)†	0·09	0·08	0·00	0·00	0·19	0·07	0·02	0·03	0·63	1·06	0·07	0·17
	Control	11	0·35	1·79	0·08	0·52	0·20	0·32	0·04	0·06	0·96	1·37	0·10	0·34
	Control	16	0·21	1·88	0·03	0·09	0·12	0·68	0·04	0·04	0·66	1·37	0·14	0·33

Results are given as net absorbance values after 10–30 min. Data on a large number of age matched controls are given in Freijd *et al.* (1984) and Hammarström *et al.* (1985).

* Different batches (with varying degrees of purity) were used. In experiments 1 and 3 batch 8 was used whereas in experiment 2 batch 7 was used.

† Age at the time of transplantation. Serum samples were obtained 2 years after transplantation.

class appear to be more protective than IgM antibodies (Briles *et al.*, 1981; Szu, Clarke & Robbins, 1983). In humans, antibodies against polysaccharide antigens are mainly of the IgG2 subclass in adults (for review see Hammarström & Smith, 1986). In children however, the response is dominated by IgG1 antibodies (Freijd *et al.*, 1984; Hammarström *et al.*, 1985; Hammarström, Persson & Smith, 1985). Since high levels of specific IgG1 antibodies against the offending micro-

organisms are present even in otitis-prone children (Freijd *et al.*, 1984) it appears as though IgG1 antibodies are less effective in conferring protection against pneumococcal infection.

In experimental animals, substantial amounts of antibodies against thymus-independent antigens such as bacterial polysaccharides may be produced in the spleen (for review see Gray *et al.*, 1984; 1985). Splenectomy in man has been suggested to lead to a lowered response to thymus-independent antigens (Hosea *et al.*, 1981; Pedersen *et al.*, 1982; Amlot & Hayes, 1985; Oldfield *et al.*, 1985), possibly as a consequence of the removal of responding cells. The impaired responsiveness, resulting in a decreased production of opsonizing antibodies, may underly the undue susceptibility to bacterial infections, observed in splenectomized patients. If a sizeable fraction of the IgG2 antibodies was to be produced in the spleen, serum levels of this particular subclass would be expected to be low in splenectomized patients. In addition, a shift of anticarbohydrate antibodies to a more immature pattern, i.e. the IgG1 subclass, could develop after challenge. However, cells originating in the spleen may seed to secondary sites as a consequence to antigenic exposure already prior to splenectomy (Amlot & Hayes, 1985) and total concentrations would then be only marginally affected. The demonstration in this paper of normal serum levels of IgM and IgG2 and an expected IgG subclass restriction pattern of anti-carbohydrate antibodies in patients with congenital asplenia and bone-marrow transplanted patients splenectomized before grafting, clearly suggests that the spleen does not to any major degree contribute to the normal background antibody repertoire. This suggestion is also supported by previous findings of a large fraction of IgG2 producing cells in the bone-marrow (Morell *et al.*, 1975), a site which is thought to be the major source of serum IgG. A note of caution must however be added since the degree of splenic involvement may differ between various pneumococcal serotypes and it is therefore posssible that splenic antibody production against pneumococcal types other than those tested may be of clinical importance. However, the serotypes investigated (3, 6A, 19 and 23) are considered to be among the main pathogens in normal individuals and may also be anticipated to contribute markedly to the spectrum of infections in asplenic patients.

Although protective levels of anti-pneumococcal antibodies have not unambigously been determined, the observation of comparable levels of specific anti-capsular antibodies (as judged by similar absorbance levels in ELISA) in normal and asplenic children would tend to suggest that acceptable levels of specific antibodies are present in the latter. Although the necessity for the involvement of spleen cells in the rapid increment in anti-pneumococcal antibody levels seen in the early phases of bacterial infection cannot be ruled out, the observed data suggest that the main contribution of the spleen in the defence against infections is its phagocytic capacity.

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